

EFFECTS OF INTRASEPTAL INJECTIONS OF
L-GLUTAMATE AND GABA ON THE RAT'S
ACOUSTIC STARTLE RESPONSE

CENTRE FOR NEWFOUNDLAND STUDIES

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OLUSOLA A. ELABANJO



EFFECTS OF INTRASEPTAL INJECTIONS OF L-GLUTAMATE AND
GABA ON THE RAT'S ACOUSTIC STARTLE RESPONSE

BY



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ABSTRACT

The specific hypothesis that cell bodies in the medial septum-diagonal band (MS-DB) complex are responsible for startle amplitude modulation was tested in rats using L-glutamate and GABA as the cell-specific excitant and inhibitor respectively.

In a series of experiments, various concentrations and volumes of L-glutamate and GABA were injected into the MS-DB complex, and the startle amplitude measured by administering 80 trials of 70 db white noise background and 110 db white noise, as the startle stimulus. L-glutamate studies revealed that injection of 1 μ l, 1 M, significantly attenuated the rat's acoustic startle response (ASR), while the GABA studies showed no difference from the control groups. The results were interpreted to have indicated that the cell bodies in the MS-DB complex are involved in the attenuation of the rat's startle amplitude, while the failure of GABA to achieve the opposite effect was probably due to its uptake and/or conversion to glutamate.

Hippocampal EEG recording taken from the rats injected with L-glutamate and GABA showed that the effects seen in the present studies were not due to the injected

drugs causing epileptiform-like discharges in the
hippocampus.

Key words : Acoustic startle response(ASR) - EEG - GABA -
Hippocampus - Intraseptal - L-GLUT. - MSB -
Septo-hippocampal - Picrotoxin

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This work is dedicated to my family for their long-distance support; to the good people in Canada that I have been fortunate to come across, without whose moral support, all of this might not have been possible. This work is also dedicated to those not so nice people, who through their actions have only succeeded in strengthening my resolve to succeed.

Table of Contents

| | Page |
|---|------|
| Introduction. | 1 |
| Habituation. | 2 |
| Acoustic Startle. | 8 |
| The Septo-Hippocampal Connections. | 24 |
| Methods. | 35 |
| Glutamate Injections. | 44 |
| GABA Injections. | 62 |
| Discussions. | 69 |
| Glutamate. | 69 |

| | |
|---|----|
| GABA. | 70 |
| Suggestions for Further Experiments. | 72 |
| References. | 76 |
| Appendix A. | 86 |

List of Tables

| Tables | Page |
|--|------|
| 1. Experiment I: Startle amplitude analysis of 1.5 μ l injections in the .5 M GLUT, 1 M GABA, and SAL. groups (using the 21st-25th trials from the separate day baseline). | 47 |
| 2 Experiment I: Startle amplitude analysis of 1.5 μ l injections in the .5 M, 1 M GABA, and SAL. groups (using the 16th-20th trials from the test day as baseline). | 49 |
| 3 Experiment II: Startle amplitude analysis of 1.5 μ l injections in the .5 M GLUT and SAL. groups. | 52 |
| 4 Experiment III: Startle amplitude analysis of 1.5 μ l injections in the .5 M, 1 M GLUT, and SAL. groups. | 56 |
| 5 Newman-Keuls analysis of the means of groups | |

| | | |
|---|---|----|
| | in experiment III. | 58 |
| 6 | Experiment IV: Startle amplitude analysis of .5 μ l injections in the .5 M, 1 M GLUT, and SAL. groups. | 60 |
| 7 | Experiment V: Startle amplitude analysis of 1 μ l injections in the 1 M, 1.5 M GABA and SAL. groups. | 63 |
| 8 | Experiment VI: Startle amplitude analysis of .5 μ l injections in the 1.5 M, 2 M GABA and SAL. groups. | 66 |

List of Figures

| Figure | Page |
|--|------|
| 1 Photograph of the startle box, showing the accelerometer resting on the adjustable pedestal. | 37 |
| 2 Means of the startle amplitudes of rats in Experiment I (using the 21st- 25th trials from the separate day baseline), expressed as percentage of baseline (n=10). | 48 |
| 3 Means of the startle amplitudes of rats in Experiment I (using the 16th-20th trials from the same baseline-test day), expressed as a percentage of baseline (n=10). | 50 |
| 4 Means of the startle amplitudes of rats in Experiment II, expressed as a percentage of baseline (n=11). | 53 |
| 5 Means of the startle amplitudes of rats in | |

| | | |
|----|---|----|
| | Experiment III, expressed as a percentage of baseline (n=9 for .5 M and 1 M GLUT groups, n=8 for SAL. group). | 57 |
| 6 | Means of the startle amplitudes of rats in experiment IV, expressed as a percentage of baseline (n=7). | 61 |
| 7 | Means of the startle amplitudes of rats in experiment V, expressed as a percentage of baseline (n=10). | 64 |
| 8 | Means of the startle amplitudes of rats in experiment VI, expressed as a percentage of baseline (n=9). | 67 |
| 9 | Schematic diagrams of internal cannula tip sites of rats used in all the analyses. | 68 |
| A1 | EEG recordings from .5 M GLUT and SAL. rats, given .5 μ l injections. | 91 |
| A2 | EEG recordings from 1 M GLUT and SAL. rats, | |

| | | |
|----|--|-----|
| | given 1 μ l injections. | 92 |
| A3 | EEG recordings from 2 M GLUT and 1 M GABA rats, given .5 μ l injections. | 93 |
| A4 | EEG recordings from 2 M GABA and 4 M GLUT rats, given .5 μ l injections. | 94 |
| A5 | EEG recordings from saline controls for picrotoxin injections, given in 1 μ l injections. | 98 |
| A6 | EEG recordings from 6 mg/ml picrotoxin rats, given 1 μ l injections. | 99 |
| A7 | Schematic diagrams of the extent of recording electrode penetrations in EEG rats. | 100 |

List of Appendices

| Appendix | Page |
|---|------|
| A Effects of septal injections of glutamate, GABA, and picrotoxin, on hippocampal EEG. | 91 |

Introduction

The acoustic startle response(ASR) of the rat is a reflexive reaction to a sudden intense auditory stimulus and is characterized by a short latency, usually between 8-14 msec, gross skeletal muscle contraction (Marsh, Hoffman, and Stitt, 1973). Landis and Hunt (1939) have described the temporal characteristics of startle in humans. The first element described was the eyeblink, with both eyes reacting simultaneously and the general pattern of the startle being eyelid closure, mouth, head and neck movements which also occur roughly together. Depending on the individual and the intensity of the stimulus, the bodily pattern becomes more noticeable with the occurrence of the general flexion which they described as " a protective contraction or shrinking of the individual ". The same basic pattern was described for infrahuman primates even in three monkeys that had previously undergone cortical ablations, suggesting that the cortex probably does not contain the basic circuitry mediating this reflex. This suggestion was further confirmed in studies with decerebrate rats, where complete transections of connections between the forebrain and midbrain(Davis and Gendelman, 1977) and between the forebrain and the hindbrain(Fox, 1979) failed to abolish

the acoustic startle and its habituation.

Habituation:

As with most reflexes, temporally close repetitive elicitations of this response result in occurrence of habituation. Habituation has been described as the gradual decrease in responsiveness, produced by repeated application or presentation of the same stimulus (Harris, 1943). Habituation is not only found in the startle response, it can also be found in most behaviours, overt or neural (e.g., the gill-withdrawal reflex in the *Aplysia*, Kandel and Schwartz, 1985), that are prone to being repeated over a period of time. Rats have a natural aversion to a new type of food, but after being exposed to it for sometime, this ingestional neophobia is readily overcome. Alcohol and drug tolerance in humans also displays habituation (Seigel, 1983). When the effects of drug administration decline with repeated use, habituation to the primary drug effects takes the form of tolerance. With these and other studies, the pervasiveness of habituation has been well documented in the literature.

Neuronal Mechanism of Habituation:

Occurrence of habituation depends on many conditions, and its parametric characteristics have already been discussed at length in Thompson and Spencer (1966), and Hinde (1970). With its amenability to analysis in *Aplysia*

californica, habituation has been found to be a process that involves changes at the neuronal level. In fact, habituation of the gill-withdrawal reflex in *Aplysia* was found to have been due in part, to a prolonged inactivation of calcium channels, resulting in decreased neurotransmitter output at the sensory neuron terminals (Castellucci, Pinsker, Kupfermann, and Kandel, 1970; Kandel and Schwartz, 1985). By immersing an *Aplysia* sensory neuron, L7, in a high Mg^{2+} , high Ca^{2+} solution, Castellucci and Kandel (1976) were able to increase the average amplitude of this sensory neuron's PSP, and this increase was reported to have lasted as long as 50mins. But because a high extracellular Ca^{2+} level was required to increase the monosynaptic output at the L7-sensory neuron terminal, Farley and Alkon (1985) questioned whether this might be applicable to a situation where physiological Ca^{2+} level was operating.

Characteristics of Habituation:

The intensity of habituation has been shown to depend on the frequency of exposure to the stimulus, and how long the training phase lasted (Davis, 1970). This habituation has been shown to persist longer than the period of stimulus administration, and this persistence has been called the retention of the habituation. Habituation and its retention have been suggested to be forms of learning

and memory respectively (Kandel and Schwartz, 1985), and like memory(short- and long-term), habituation has been broadly divided into two aspects: 1) Long-term(between session) habituation, and 2)Short-term(within session) habituation. As in the case of sensitization and habituation, studies have shown that two different processes are in operation for these two types of habituation. Exposing rats to 1000 tones of 120 db intensity, at interstimulus interval(ISI) of 2 and 16 secs, and then measuring their startle response 1 min. or 24 hrs later, Davis (1970) reported greater habituation for the 16 sec ISI rats than for the 2 sec ISI rats, suggesting that these two types of habituation may be distinguishable.

The categorization of habituation into long- or short-term depends on the species, the behaviour, and how the behaviour was measured. Long-term habituation has been described as the persistence of habituation over a period of time. In ASR habituation, this period of time has ranged between 24 hrs (Groves, Wilson and Boyle, 1974) and 48 hrs (Jordan and Leaton, 1982b) between test sessions. Another aspect of long-term habituation which has been labelled as the retention phase, particularly in ASR, has also been tested for, days after the initial habituation. Jordan and Leaton (1982) tested for retention at 6 days, while Miller and Treft (1979) tested

for retention at 10 days after the last test day.

Different structures appear to underlie the different forms of habituation. Damage to the mesencephalic reticular formation (MRF) has been shown preferentially to disrupt the long-term habituation of the ASR (Jordan and Leaton, 1982a), over the short-term habituation of the same response. By the same token, vermal lesions have also been reported to block the acquisition of long-term habituation of the ASR in rats preferentially (Leaton and Supple, Jr. 1985). These and other studies have conclusively shown that the behavioural and neural processes operating in both types of habituations are distinguishable, and therefore different.

Attempts have been made to elucidate the cellular mechanism underlying long-term habituation. Carew and Kandel (1973) studied an isolated ganglion preparation from the L7-sensory neuron in *Aplysia*. Acquisition of long-term habituation was reported to have been associated with progressive decrement in the complex EPSP produced in L7 by stimulation of the afferent nerve. Castellucci, Carew and Kandel (1978) also using isolated ganglion preparation from *Aplysia*, reported a lower incidence of detectable synaptic connections for experimental against control groups, in sensory neuron-L7 connections. They also reported diminished transmission across this synapse 24hrs. after the first test. On the other hand, Carew,

Castellucci and Kandel (1979) were able to reverse this long-term habituation with a single sensitizing stimulus. This rapid reactivation made them conclude that gross morphological changes in the synapse may be ruled out as part of the mechanism underlying long-term habituation in *Aplysia*.

Brain Sites Involved:

Though studies have shown that forebrain structures are not essential for habituation of the ASR, it could be said that they may participate in the process, hence the need for a brief review of studies on forebrain structures that may be involved in habituation of the ASR. Studies have shown various areas of the brain to be involved in the long-term habituation to the rat's ASR. In rats that sustained bilateral, dorsal and posterior hippocampal ablation, Coover and Levine (1972) demonstrated that the hippocampectomized rats consistently exhibited higher startle amplitude, over four consecutive sessions, with no loss of habituation. However, work in this laboratory (Harley and Evans, 1982, unpublished) indicates that this may be ascribed to an alteration of sensitization. On the other hand, Leaton (1981) lesioned the anterior part of the hippocampus and after testing for acoustic startle habituation, concluded that the hippocampus is not important in the control of long-term habituation.

Decreased inhibition of the ASR was also reported in rats with MRF lesions(Jordan and Leaton, 1982a), and in rats with nucleus reticularis pontis caudalis(RPC) lesions(Leitner, Powers, and Hofmann, 1980) .

Evidence from studies in which habituation was tested following lesions of the medial septum(MS) has led to the suggestion that it might be involved in ASR habituation(Williams, Hamilton, and Carlton, 1974; Miller and Treft, 1979). In Williams et al. (1974) the medial septal(MS) lesions also included the nucleus of the diagonal band(DB), and they reported impairment of habituation. The magnitude of the startle amplitude of the lesioned rats, recorded as a percentage of the first startle, showed that the lesioned rats never attained the same level of habituation as the controls. Miller and Treft, (1979) also found impairment of long-term habituation in rats with total MS lesions. They tested the rats for habituation over four days (three consecutive days, and then 10 days later). On the first day of testing, although they found that the lesioned and control rats displayed the same rate of habituation at the about same point during stimulus presentations, the startle amplitude of the lesioned rats was significantly higher than that of the controls. On the next two days, the initial and terminal responses revealed that the control groups had displayed retention of habituation but the

total septal lesioned group still displayed significantly higher response levels. On the last test day, which was ten days after the third test, all the other groups still displayed savings of habituation, while the total septal lesion group did not. Their initial and terminal responses were similar to what they had exhibited on the first test day, suggesting loss of habituation. The Miller and Treft (1979) and Williams et al. (1974) studies came to essentially the same conclusion, that the MS is involved in habituation of the rat's ASR, though the extent of lesions in these two studies differed.

Acoustic Startle:

So far, this treatise has dealt with habituation, but with greater emphasis on ASR habituation, and since ASR habituation is an inference drawn from the temporal study of the pattern of the amplitude of individual startle responses, it is only appropriate to examine the modulation of individual startle responses, and the neural circuit mediating these responses.

Neural Circuit:

The primary neural circuit for acoustic startle in the brain stem has been documented through lesions and electrical studies at putative sites of control (Davis, Gendelman, Tischler and Gendelman, 1982; Davis, Astrachan, Kehne, Commissaris and Gallager, 1984). The first synapse

in this circuitry can be found at the posterior ventral cochlear nucleus (VCN), bilateral lesion of which completely abolishes ASR while bilateral single pulse stimulation in awake rats elicits startle-like responses, which initially sensitize, and then habituate (Davis, Parisi, Gendelman, Tischler and Kehne, 1982). Direct projections from the VCN synapse in the dorsal (DLL) and ventral (VLL) lateral lemniscus (Harrison and Irving, 1966). The next synapse is at the nucleus reticularis pontis caudalis (RPC), lesions of which abolish the ASR and bilateral electrical stimulation at certain frequencies elicits increased startle-like responses in awake rats. Axons emanating from this area project to all levels of the spinal cord through the reticulo-spinal tract (RST), which passes near or through the medial longitudinal fasciculus (MLF), and then divides into two to form the ventral funiculi in the spinal cord (Grillner, Hongo and Lund, 1971; Peterson, 1979; Peterson, Maunz, Pitts and Mackel, 1975). The short latency (8 msec) of the hindleg startle response is consonant with the relatively few synapses of the primary acoustic startle circuit.

Modulation by Drugs:

With a startle circuit elucidated, the question then arises as to why the interest in this response itself? It could be argued that the startle response is part of a

defensive reaction, and that this is a generalized reaction across species. The first few stimuli in a startle test may be serving to increase the attention of the organism concerned to a novel stimulus (particularly an acoustic stimulus in mammals), so as to prepare the organism for a fight or flight situation, if necessary. This response then habituates if the stimulus is perceived by the organism as non life-threatening. Other pertinent questions to ask are: how does this reflex or response relate to other behaviours, and how would it help us understand some of the higher level functions, or guide us to some abnormalities in their functioning?

The startle response has been found to be a versatile tool for investigating reactions to different kinds of drugs because of the simplicity of its basic neural circuit and the ease with which plasticity can be displayed in the behaviour itself. The effects of hallucinogens on the ASR has been investigated by giving intraperitoneal(ip) injections of psilocybin to rats. Davis and Walters(1976) reported a dose-dependent, biphasic response in rats. At low doses (0.5-1 mg/kg), psilocybin elevates startle amplitude while depressing it at higher doses (2-8 mg/kg). They concluded that the elevation of startle amplitude at low doses was a result of selective inhibition of the firing rates of the midbrain raphe neurons, while the depression at higher

doses was as a result of the drug diffusing to neurons postsynaptic to these raphe cells, and inhibiting them. The tricyclic antidepressants, chlorimipramine (CIMI), desipramine (DMI), imipramine (IMI), and chlordesipramine (C-DMI), were reported to block the excitatory effects of the low doses of a hallucinogen (LSD), on startle amplitude (Davis, Gallagher and Aghajanian, 1977). They suggested that except for DMI and C-DMI, the tricyclics achieve this blocking action by indirectly inhibiting the firing rate of the midbrain raphe neurons, which is the same mechanism by which the hallucinogens augment startle amplitude. This indirect inhibition by the antidepressants is achieved by blocking the reuptake of 5-HT. Commissaris and Davis (1982) found opposite effects of systemically administered N,N-Dimethyltryptamine (DMT) and 5-Methoxy-N,N-Dimethyltryptamine (5-MeODMT), with DMT suppressing and 5-MeODMT enhancing the acoustic startle amplitude. They suggested that the opposite effects of these two drugs are mediated by their actions on 5-HT receptors in the brain and spinal cord respectively.

Modulation by the Environment:

Changes in the visual or auditory environments have been reported to affect startle amplitude. In a series of experiments using light or sound prepulses (a stimulus

that precedes the startle stimulus), of three different intensities Ison and Hammond (1971) reported that there was a positive correlation between inhibition of startle amplitude, and the applied prepulse intensities in both modalities. They also reported that facilitation of startle amplitude was positively correlated to stimulus intensities of the visual stimulus, while exhibiting a nonmonotonic relationship in the auditory modality. Groves, Boyle, Welker, and Miller (1974) suggested this startle amplitude inhibition by sound prepulse to be due to the reflexive contraction of the middle ear muscles (stapedius and tensor tympani), on the evidence that the intensity of reflexive contraction of the middle ear muscles have been shown to be directly proportional to the intensity of the sound prepulse (Carmel and Starr, 1963; Hoffman and Searle, 1965). Groves et al. (1974) administered bilateral electrolytic lesions to the motor nuclei of the fifth and seventh cranial nerves, which control the reflexive contraction of the middle ear muscles, and reported an impairment of the auditory prepulse inhibition of the ASR in rats.

Changes in the background white noise have been shown to modulate the rat's acoustic startle amplitude. Hoffman and Fleshler (1963) reported that, in contrast to pulsed background noise which significantly depressed startle amplitude, steady background noise increased it. This

enhancement of startle can be increased if the animals had a prior exposure to the white noise, Davis (1974b). He reported that with a 5-30 min prior exposure of rats to white noise, there was an increase in startle amplitude, which continued to grow for at least 30 min, and took 15 min. after the noise was turned off to dissipate. Various levels of background white noise have also been shown to have different effects on startle amplitude, depending on whether it occurred before or during startle. Increasing the level of background noise at the onset of startle elicitation, Hoffman and Wible (1969) reported that within 100 msec of startle elicitation, there was a facilitation in its amplitude, which became stable and then abruptly declined when the noise was turned off. Eliciting startle with a 110 db-tone in the presence of 60, 70, and 80 db white noise backgrounds, Davis (1974a) reported an inverse c. a nonmonotonic relationship to the noise, whereas Davis (1974b) reported a direct relationship between startle amplitude and the intensity of the noise to which the subjects were preexposed. These studies show that very slight changes in the environment prior to or during testing can affect the magnitude of the rat's ASR.

Modulation by Transmitters:

If changes in the rat's immediate external

environment could affect the magnitude of the startle amplitude, then changes in the rat's internal environment should be expected to alter startle amplitude. Lesion (discussed above), and/or pharmacological agents have not only been shown to alter the magnitude of the startle amplitude, but at the same time, they have also pointed out the probable neurotransmitters likely to be involved in the modulation of the startle amplitude. By manipulating the effectiveness of the different transmitters, particularly dopamine, norepinephrine, serotonin and acetylcholine, it has been possible to demonstrate the involvement of these transmitters either in the sensitization or habituation of the startle response, and hence the startle amplitude also.

The role of dopamine (DA) in the modulation of acoustic startle has been extensively investigated with ip injections of agonists and antagonists to this transmitter. Apomorphine, which is widely accepted to have DA agonistic properties, has been reported to elevate startle amplitude (Davis and Aghajanian, 1976; Kehne and Sorenson, 1978), and this effect occurred between 2-4 min after injection. Apomorphine's effect was blocked by the DA antagonist haloperidol, in doses that did not produce significant depressant effects on startle itself. The potent catecholamine agonists, d- or l-amphetamine, markedly increased acoustic startle in rats (Kehne and

Sorenson, 1978; Kirkby, Bell and Preston, 1972; Davis, Svensson and Aghajanian, 1975). The doses used in these studies were in the same range as those required to produce stereotypic behaviours in rats, which are known to be mediated by DA transmission. This startle amplitude potentiation by amphetamine should not be viewed as an artefact of increased motor activity, because most startle devices are designed to filter out motor movements that were not due to startle. The above mentioned potentiating effect of amphetamine was blocked by pretreatment with pimozide, a potent DA receptor blocker, or with alpha-methyl-p-tyrosine, a tyrosine hydroxylase inhibitor, (Kehne and Sorenson, 1978). Depletion of central DA levels by 200 ug ip injection of 6-OHDA resulted in elevated startle response (Sorenson and Davis, 1975). Furthermore, pretreatment with reserpine also elevated startle response. Fechter (1974) administered 5 mg/kg of reserpine to rats and then 100 mg/kg of MK-486, a peripheral L-amino acid decarboxylase inhibitor, six hours later. Seventy min after MK-486 administration, they were either given saline or 100 mg/kg L-dopa and then tested 40 min later. A significant startle elevation was reported from reserpine treatment, with L-dopa restoring their amplitudes to "normal" levels. The report of this and the Sorenson and Davis (1975) studies suggests an inhibitory role for the DA system. In a review, Davis (1980) pointed

out that the confusion might have arisen from the choice of the interval between injection and testing. This is because apomorphine has a biphasic effect on startle (Davis and Aghajanian, 1976), so depending on the injection-test interval, an elevation or depression of startle may be seen. With apomorphine, startle was increased 5-40 min after injection and then a depression phase set in (40-80 min), so the effect of L-dopa reported in Fechter (1974) might have been due to the depressant phase of the drug, since L-dopa has been shown to have biphasic effects on spinal reflexes (Commissiong and Segdwick, 1974), and testing in that study did not take place until 40 min after L-dopa administration. Despite a few contradictions, it is still widely held that increases in activity at DA synapses increase the ASR but whether the same holds true for its opposite is uncertain.

The role of norepinephrine (NE) in startle amplitude modulation is even less certain than that of DA. Kehne and Sorenson (1978) put forward the most convincing evidence so far, for the role of NE in startle modulation. They reported that ip injections of phenoxybenzamine, an alpha-adrenergic receptor blocker, completely blocked the potentiating effects of high doses of apomorphine and l-amphetamine, but not d-amphetamine. Since both isomers elevate startle amplitude, this suggests that there were two mechanisms. Clonidine, a known inhibitor of the

activity of locus coeruleus (LC) NE, by activating alpha-2 receptors, depressed startle amplitude, with the effect being monotonically related to the dosage used (Davis, Cedarbaum, Aghajanian, and Gendelman, 1977). This effect of clonidine was also reported to have been antagonised by pretreatment with piperoxane but not phentolamine. Davis et al. (1984) showed that the depletion of central and peripheral NE by the neurotoxin, DSP-4, completely inhibited the excitatory effects of yohimbine on startle amplitude. This effect can be counteracted by prior (30 min) pretreatment with desipramine, an NE uptake blocker. These studies demonstrated that activation of alpha-2 adrenergic receptors depress startle amplitude.

Intrathecal administration of 50 µg phenylephrine (PE), an alpha-1 adrenergic agonist, was reported to have enhanced startle amplitude (Astrachan and Davis, 1981), by acting on alpha-1 adrenergic receptors in the spinal cord. This was substantiated by pretreating rats with 6-OHDA or its vehicle and then testing one week later, with various doses of PE (12.5, 25, and 50 mg). They reported that intrathecal administration resulted in startle amplitude enhancement that was directly related to the amount of PE injected. Furthermore, evidence to support the alpha-1-adrenergic theory was obtained from assay binding studies. Infusing rats with either 6-OHDA or its vehicle and then intrathecally injecting them with various doses

of PE, Astrachan, Davis, and Gallager (1983a,b) reported that lower doses of 6-OHDA were more effective in enhancing startle amplitude in response to PE, suggesting supersensitivity at the receptor sites. Subsequent lumbar cord assay using 3H-prazosin as the specific high-affinity alpha-adrenergic ligand showed a larger number of 3H-prazosin binding sites in 6-OHDA treated animals than in vehicle treated ones.

Intraperitoneal administration of clonidine significantly depressed acoustic startle (Davis et al., 1977; Fechter, 1974), and this clonidine effect was blocked by administering alpha-2 adrenergic antagonists, piperoxane or yohimbine (Davis et al., 1977; Davis and Astrachan, 1981). As was mentioned above, clonidine activates alpha-2 receptors, which are presumed to mediate the negative feedback activity of LC NE neurons. These neurons have been shown to project directly to the lumbar spinal cord. It may then be inferred from all these studies that activation of spinal alpha-1 adrenergic receptors enhances acoustic startle while activation of alpha-2 adrenergic receptors depresses acoustic startle through a negative feedback loop.

The midbrain serotonin (5-HT) system seems to have a clearer role in acoustic startle modulation than either the DA or NE systems. The general consensus in the literature is that activation of 5-HT synapses depress

startle, while thier depression or elimination enhance startle (Davis and Bear, 1972; Davis and Sheard, 1976; Geyer, Rose and Petersen, 1979; Walters, Davis and Sheard, 1979). Electrolytic lesions of the dorsal and median raphe nuclei resulted in a pronounced increase in acoustic startle (Davis and Sheard, 1974a) and because this effect was immediate(30min. after lesion), it is taken to be a result of direct 5-HT decrease and not as a result of receptor supersensitivity.

Pharmacological manipulations of this 5-HT system have also basically shown the same effect as lesioning, but in differing degrees. Depletion by parachlorophenylalanine (PCPA) (Conner, Stolk, Barchas, and Levine, 1970) was found to produce only a small increase in acoustic startle, but on the other hand, parachloroamphetamine (PCA) produced a time-dependent, biphasic reponse. PCA (5 mg/kg) given ip to rats initially depressed startle and later elevated it. This is because PCA releases 5-HT, 15-40 min after administration and thereafter causes a long-term depletion via a neurotoxic action (Sanders-Bush, Gallager and Sulser, 1974). This suggests that depending on when startle is measured after PCA administration, either a depression or an enhancement of acoustic startle may be seen.

Very low doses of halucinogens LSD, DMT and

psilocybin, increased startle (Davis and Sheard, 1974b; Davis and Walters, 1977) whereas higher doses depressed it. The reason is that low doses decrease 5-HT release by decreasing impulse flow while higher doses, (though doing the same thing as the low doses), also act as agonists by depressing the firing rates of the post-synaptic neurons, hence mimicking 5-HT action (Davis and Walters, 1976). Davis (1980) also suggested that these hallucinogens modulate startle amplitude via the sensory and not the motor side of the reflex arc. This suggestion would fit with the role of "the filter" that had been ascribed to the 5-HT systems in humans (Snyder, 1980). The use of psychedelic or hallucinogenic drugs by humans has been suggested to cause "a change in the filtering mechanisms, so that normally screened out features of the external environment and repressed internal feelings now penetrate into awareness" (Snyder, 1980). Apart from its other actions, small doses of LSD are known to inhibit 5-HT neurons in the brain (Aghajanian, Foote and Sheard, 1973). Assuming that hallucinogens modulate startle on the sensory side of the reflex arc, it may be suggested that inhibition of the serotonin systems could lead to inhibition of the "filtering system" and hence allowing "normally screened out features of the external environment" to pervade "awareness" and therefore elevate the startle amplitude.

The role of the cholinergic system in startle modulation has been extensively investigated and reported in Overstreet (1977). In this study, he injected rats ip with physostigmine, atropine, pilocarpine, and diisopropylfluorophosphate (DFP), an anticholinesterase agent. He reported that DFP did not affect the habituation of startle, after tolerance to the drug had developed, suggesting that impairment of habituation caused by intramuscular injection of DFP is only a short-lived phase. How long it lasted depended on when tolerance to the drug was developed. Physostigmine did not alter the rate of habituation, but atropine (5 mg/kg) was reported to have increased the startle amplitude over the first few trials but habituation developed later. This habituation seemed to have started by the ninth trial. Other dosages of atropine (1, and 10 mg/kg) used in the experiment did not increase startle amplitude as much as the 5 mg/kg dosage. This was attributed to the large individual differences within the groups. On the other hand, depression of startle amplitude was reported for all dosages of pilocarpine (1, 5, 10 mg/kg). Habituation also occurred as long as the dosage was not high enough to have reduced the initial startle amplitude to the level of the asymptote seen at the end of habituation of acoustic startle. These findings led him to conclude that the cholinergic system has little or

nothing to do with startle response habituation, but only affects the basal startle response amplitude. He went on to conclude that drugs that raise the levels of Acetylcholine (ACh) or mimic its action lower the response level, while those which block the muscarinic action of ACh raise the response level.

Modulation by Lesions:

Lesion (electrolytic, knife cuts or otherwise) studies have been used to study many different behaviours, with the ASI in rats being no exception. Lesions of the RPC and not the nucleus reticularis gigantocellularis (RGI) produced a decrease in startle response amplitude (Leitner, Powers and Hoffman, 1980) leading to the conclusion that this structure is involved in the elaboration of the mammalian startle response (Hammond, 1974; Leitner et al. 1980; Szabo and Hazafi, 1965). Lateral tegmental lesions (Leitner, Powers, Stitt and Hoffman, 1981) produced a decreased inhibition to acoustic prestimuli (prepulse), showing the involvement of this area in the mediation of startle inhibition due to acoustic prestimuli (prepulse).

Jordan and Leaton (1982a), found that lesioning the mesencephalic reticular formation (MRF) resulted in the disruption of long-term habituation with apparently no effect on the short-term component. On the other hand,

Jordan and Leaton (1982b), lesioned the brachium of the inferior colliculus (BIC), and reported no effect on either of the two components of habituation but found that this lesion resulted in an increase in startle amplitude.

Hippocampectomized rats consistently exhibited higher startle amplitude than controls, over four consecutive sessions (Coover and Levine, 1972), indicating that this area exerts some form of tonic inhibitory influence on the primary acoustic startle circuit. Another area of the rat's brain that has been implicated in modulation of ASR habituation is the MS, which is rostral relative to the hippocampus. Lesioning the MS (Williams et al., 1974) resulted in startle amplitude during initial presentations of the stimulus being significantly higher than that for controls. This same conclusion was reached by the Miller and Treft (1979) study. Looking back on the Coover and Levine (1972) conclusion that the hippocampal exertion of tonic inhibitory influence on the acoustic startle, and the McNaughton and Miller (1984) finding that stimulation of the MS activated dentate granule cells, it could be said that both the inhibitory influence and the message for dentate activation may have been generated in the MS, and then relayed to the hippocampus through the septo-hippocampal (SH) connections.

THE SEPTO-HIPPOCAMPAL(SH) CONNECTIONS:

Some of the earliest studies that demonstrated a probable connection between the septum and the hippocampus showed that the rabbit's hippocampal rhythmical activity depended on the periodicity of septal pulse density arriving at the hippocampus (Petsche, Stumpf and Gogolak, 1962; Stumpf, Petsche and Gogolak, 1962). Gogolak, Stumpf, Petsche and Sterc, (1968) went on to superimpose the rabbit's septal population discharge on the hippocampal theta rhythm graphically, and they reported a similarity between the two curves. This suggests that the theta rhythm may be determined by the frequency distribution of the firing of the septal units, and that there are connections between the MS and the hippocampus. Since then, other studies (e.g., Mosko, Lynch, and Cotman, 1973; Krnjevic and Ropert, 1981; Krnjevic and Ropert, 1982) have also demonstrated the existence of these connections. In a recent study Collins and Crutcher (1985), using conditioned medium prepared from slices of the rat hippocampus, showed that the level of activity of a nerve growth factor (NGF)-like growth promoting factor was significantly increased in the dentate gyrus-CA3 and CA1 regions within one week of MS lesions. This level of activity was maintained for at least 4 weeks after the lesions, whereas control lesions that failed to interrupt the SH innervation did not cause an increase in levels of

activity in the SH formation. This study further confirmed the presence of the SH connections and one of the effects of their absence, on parts of the hippocampus.

Efforts have been made to investigate the nature of the septum, the hippocampus, and the connections between them. Some studies have shown that these connection are mostly cholinergic (Mosko, Lynch and Cotman, 1973; Mellegren and Srebro, 1973; Crutcher, Madison and Davis, 1981). Clarke (1985) used a monoclonal antibody to choline acetyltransferase (CHAT) and reported dense fiber networks at the interface of granule cell layer and molecular layer. With the aid of horseradish peroxidase (HRP) injection into the MS, Chandler and Crutcher (1983), were able to show dense labelling in the rat's dentate gyrus. This finding has been confirmed by immunocytochemical and electron microscopical study. Apart from the dentate and molecular layers, the SH fibers have also been shown to project to other areas of the hippocampus. Sainsbury and Bland (1981) have shown that selective septal lesions disrupted theta production in the CA1 and the dentate gyrus. The septal connections responsible for producing CA1 theta pass through the fornix while those that mediate dentate theta course through the fimbria (M'Harzi and Monmaur, 1985). Fornix lesions mainly affected CA1 theta, fimbrial lesions affected only dentate theta, while combined fornix-fimbria

lesions suppressed both the dentate and CA1 thetas.

Blaker, Peruzzi and Costa (1984, suggested that the MS projects to the hippocampus in a topographical manner. They went on to suggest that the dorsal hippocampus is innervated by midline medial septal neurons which are presumably cholinergic, and that the ventral hippocampus is innervated by neurons in the lateral aspect of the MS and also that these neurons are presumably GABAergic. Application of muscimol, a GABA agonist, to these GABAergic MS neurons resulted in attenuation of hippocampal theta wave (Allen and Crawford, 1984), suggesting that the MS may be an important pacemaker driving the hippocampal theta and this function may be subject to GABA-like actions.

By the use of fluorescent tracer histology, combined with choline-O -acetyltransferase (ChAT) immunohistochemistry and AChE histochemistry, Woolf, Eckenstein and Butcher (1984) were able to demonstrate cholinergic projections from the MS-DB to the hippocampus. Acetylcholine (ACh) has been shown to be a transmitter in the hippocampus (Nicoll, 1985) and paired-pulse stimulation of the ventral portion of the MS (in this case the DB) after ip injections of physostigmine resulted in an increase of amplitude of a late positive component of the average evoked potential recorded from CA1 pyramidal cells, elicited by the second stimulus of a pair

(Hettinger and Gonzalez, 1984). This enhanced response was similar to that seen in pyramidal cells after septal stimulation. These studies show that the SH connections are mainly cholinergic, and that ACh is probably used as a transmitter in the hippocampus.

On the other hand, other studies have shown that the SH projections are not mainly cholinergic. In fact, with the aid of a double-label HRP-AChE study in the rabbit, Baisden, Woodruff and Hoover (1984) reported that less than 50% of the neurons in the MS and DB which were labelled by HRP injection into the dorsal hippocampus also stained for AChE. They suggested substance P (SP) as another possible transmitter for the non-cholinergic neurons. Evidences for SP receptors in the MS-DB was provided by Lamour, Dutar and Jobert (1984) by antidromical electric stimulation of the fimbria. Iontophoretic application of SP onto SH neurons resulted in strong excitation comparable to that often seen with carbachol administration. Employing the autoradiographic analysis of the binding of [125 I] Bolton-Hunter coupled SP ([125 I]BH-SP), Rothman, Herkenham, Pert, Liang and Cascieri (1984) were able to visualize SP receptors in the external portion of the DB. Immunocytochemical methods have also been used by Gall and Moore (1984) to show that there is SP-like immunoreactivity in the septum and also 5-HT- and tyrosine hydroxylase (TH)-like immunoreactive axons were seen to be

coursing through the MS-DB complex, without any evidence of terminal varicosities.

THE MEDIAL SEPTUM:

Modulation of a response is generally taken to be an alteration in the response topography. Habituation describes one particular form of modulation: a gradual decrease over trials, in the amplitude or frequency of a response. Sensitization, though an opposite of habituation, is yet another form of response modulation.

The Miller and Treft (1979) finding that septal lesions impaired habituation of the ASR in rats is important to the present study, because their result suggests that the removal of cholinergic and other projections from the MS to the hippocampus, by MS lesions, might have resulted in the impairment of habituation seen in their study. Unfortunately, the electrolytic lesions had the effect of totally removing all cell bodies, terminal boutons, and even axons of passage. So the effect of these lesions reported in their study might have been due to the absence of any or a combination of all these elements in the MS.

The septum is made up of the medial, lateral, posterior, and ventral divisions. Because of the nature of the present study, this brief anatomical and physiological review will restrict itself to the MS and

its association with the hippocampus. The MS is conveniently divided into medial septal nucleus dorsally, and the nucleus of the diagonal band of Broca ventrally. Since the Williams et al., (1974) and Miller and Treft (1979) studies arrived at basically the same conclusion though one lesion included the diagonal band and the other did not, and also since the present study will not be distinguishing between the MS and the DB, then for the purpose of this study alone, both areas shall be collectively referred to as the medial septal body (MSB), when being referred to together, or the medial septum (MS) alone and nucleus of the diagonal band (DB) alone.

Some Cell Groups in the MSB:

The MSB receives its major input from the lateral septum (Costa, Panula, Thompson and Cheney, 1983) and then projects through the dorsal fornix, fimbria (M'Harzi and Monmaur, 1985), and probably the cingulum to the hippocampal formation. Other ascending inputs to the MSB arise in several hypothalamic nuclei and the brain stem aminergic cell groups. Costa et al., (1983) stated that the MSB contains either cell bodies and/or terminals that release a variety of transmitters including GABA, ACh, DA, NE, glutamate, SP, beta-endorphin, and met-enkephalin, showing that the MSB contains many different sub groups of cells. Only a few of these putative transmitters will be

reviewed here.

Acetylcholine:

There are cholinergic cell bodies in the MSB which project to the hippocampus and evidence to back this claim has already been discussed above. If the cholinergic projections to the hippocampus are altered indirectly either by blocking or activating one of the neurotransmitters in the MSB during startle, then this should be reflected in the startle amplitude. Bilkey and Goddard (1985) suggested that most of the septal projections to the dentate gyrus are GABAergic, which act to inhibit interneurons in the hippocampus. The Baisden et al. (1984) study also reported that many of the MSB-hippocampal projections are non-cholinergic, and they suggested substance P (SP) as another possible transmitter.

Substance P:

The MSB contains SP terminals (Costa et al., 1983). Blaker et al. (1984) found that intraseptal (specifically MS) injection of SP decreased ACh turnover rate in the dorsal but not in the ventral hippocampus. They suggested that cholinergic projections to these two hippocampal areas are mediated by different transmitter systems and hence have different physiological functions.

Using immunocytochemical methods, Gall and Moore

(1984) found that about 50-100 ug colchicine injected intraventricularly, 48hrs prior to sacrifice, revealed SP-immunoreactive perikarya in the septum. They also found that this reactivity was only localized within axons outside the MSB. This could be interpreted to suggest that the SP neurons projecting from the lateral septum, synapse on ACh neurons in the MSB to produce their effects on ACh turnover rate in the hippocampus. With autoradiography, Rothman et al. (1984) were able to visualize SP receptors in the external portion of the DB. Their autoradiographs showed that these receptors were confined to the very ventral part of the DB, suggesting that these receptors could be autoreceptors on the terminal boutons of the SP axons projecting from the lateral septum to the DB. These two studies taken together, suggest that SP cells are located outside the MSB, but they send axons into the MSB which probably synapse on ACh neurons located in this area.

Norepinephrine:

In a review, Costa et al. (1983) discussed the locus coeruleus (A6) projections to the MSB, while a medulla oblongata noradrenergic system originating in the A1, A2, or A3 cell groups projects to the lateral septum and the DB. Costa et al. (1983) also went on to suggest that NE release from septal noradrenergic terminals may

participate in eliciting an increase in turnover rate of hippocampal ACh. Their reasoning was based on the accelerated firing rate of the MS neurons, as a result of intraperitoneally injected amphetamine. Marchand and Hagino (1983) stimulated the fimbria to evoke a maximal negative field potential within the lateral septum, and iontophoretically applied NE to the lateral septum while also recording from the same place. They reported that NE decreased the activity in the lateral septum and suggested that modulation of the pace maker neurons in the MS could be achieved by way of hippocampal feedback. Projections of the hippocampal formation terminate in the lateral septum, which in turn sends projections to the MS, thereby influencing the pace makers.

Dopamine:

Dopaminergic projections to the lateral septum originate in the A10 group of cells located in the ventral medial tegmentum. It was reported (Costa et al. 1983) that when 6-OHDA was injected into A10, DA content in the septum was reduced to 32% of control without altering the NE content. Under these conditions, the ACh turnover rate in the hippocampus was increased 250%. These A10 dopaminergic neurons terminate in the medial aspect of the lateral septum and in the DB. The increase in ACh turnover rate in the hippocampus should be expected in the

light of the Stanzione, Calabresi, Mercuri and Bernadi (1984) report that iontophoresed DA onto hippocampal CA1 neurons resulted in inhibitory effects on the CA1 action potentials. Since ACh has cell bodies in the DB, Costa et al. (1983) postulated a direct interaction between the DA and ACh systems in the DB, while pointing out that an inhibitory interneuron must be interposed between the two systems for an interaction to occur in the MS. This seems reasonable in the light of the fact that there is no DA terminal in the MS. In contrast, Gall and Moore (1984) reported TH-like immunoreactive axons coursing through the MSB, but they reported no terminal varicosities there, ruling out suggestions that there may even be DA terminals in the DB itself, which will be in contrast to the Costa et al. (1983) suggestion. In summary, septal DA depletion resulted in increased ACh turnover rate in the hippocampus, but conflicting evidences on the presence or absence of DA terminals in the MSB has made it hard to determine whether the DA terminals in the lateral septum have any direct influence on ACh cells in the MSB.

The Present Study:

As mentioned above, habituation may be seen as a particular form of modulation. Thus elucidating the mechanism of response modulation, will contribute to our understanding of mechanisms of habituation.

This study proposes to look at the role of the MSB in the modulation of the rat's ASR amplitude. As discussed above, lesions have the effect of eliminating everything in the area of the lesion, so it may not be totally correct to attribute any effect seen from the lesion, to the lack of only one aspect of the area lesioned, especially in the case of the MSB. The use of finer techniques that will affect only the MSB cell bodies and not axons of passage should provide a better insight into the involvement of this area in the modulation of the rat's startle amplitude. To this end, this study has used cell-specific excitation and inhibition, by the use of L-glutamate and GABA respectively, so that only the cell bodies in the MSB would be affected by this manipulation. The rationale being that if the MSB and/or the SH connections are likely to be involved in modulating startle amplitude as suggested by the Williams et al. (1974) and the Miller and Treft (1979) studies, then L-glutamate should excite the SH connections, increasing the turnover rate of ACh in the hippocampus, and thereby depress the startle amplitude. On other hand, GABA should inhibit these connections, by decreasing the firing rate of cells in the MSB, and thereby decrease the turnover rate of ACh in the hippocampus, and in the process increasing the startle amplitude. This kind of treatment should rule out the involvement of the terminal boutons in

the MSB and also of axons coursing through the MSB, unless of course there are axo-dendritic synapses in this area, which are yet to be identified.

METHODS:

Male Sprague Dawley rats (Charles River, Canada), weighing between 250 g-300 g were anaesthetised with Sodium pentobarbital (60 mg/ml) and then positioned in a stereotaxic instrument. The incisor bar was then used to make adjustments until the vertical measures of lambda and bregma points were equal. Three holes were then drilled into the skull (one hole was drilled on the left side, rostral to bregma, while one hole was drilled on the left and right sides, caudal to bregma). These screw positions have been found to allow for sufficient anchoring of dental cement to the skull for at least three weeks.

A 22-gauge guide cannula (Plastic Products), cut to a length of 6.5 mm was then mounted on the stereotaxic arm via a small alligator clip. The perpendicularity of the guide cannula to the horizontal was verified by the use of a plumbline.

The stereotaxic arm holding the guide cannula was maneuvered until it was positioned at 0.7 mm rostral to bregma and on the midline. A pen mark was made at this point, and the stereotaxic arm was removed. A hole was then drilled on the mark to expose the dura, which was then subsequently broken with a hypodermic needle point. Three stainless steel skull screws were then put in the holes made for them, and the stereotaxic arm was then

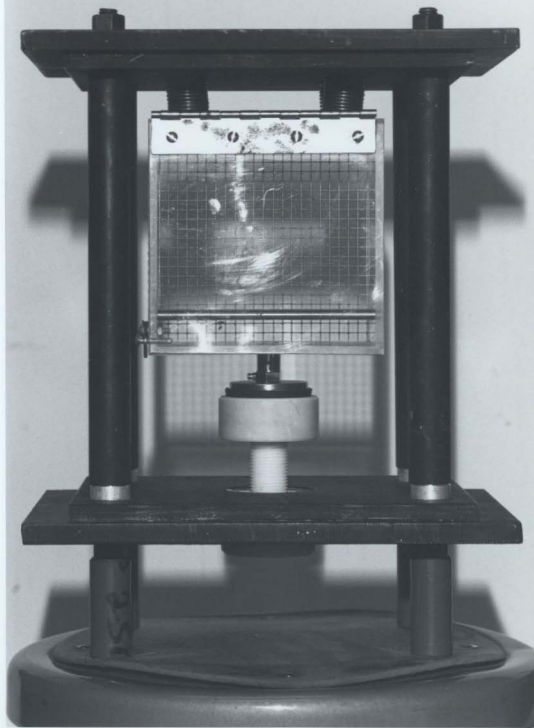
brought back to lower the guide cannula 6.5 mm below the skull surface. A 27-gauge dummy cannula, cut to a length of 7 mm was screwed into the guide cannula to prevent blockage. All subjects were individually housed in a room on a 12/12 L/D cycle, and were allowed nine days to recover from surgery.

APPARATUS:

The test chamber consisted mainly of a slightly modified Davis box (Davis and Cassella, 1984, Personal Communication) placed in a Lafayette sound-attenuating chamber, with a one-way mirror. The Davis box is a 152.4x152.4x88.9 mm (plexiglas) box, sandwiched between a model 2217E accelerometer below (Endevco, Calif.), and 4 compression springs above (fig.1). The modification was an adjustable pedestal instead of the rubber stopper on which the accelerometer sat. This pedestal allowed for the adjustment of the tension in the springs.

The output from this accelerometer was fed into a WPI-DAM6 pre-amplifier (W.P. Instruments, New Haven, Conn.) that was set between 1 Hz and the wide band pass. The pre-amplifier's output was then fed into a Krohn-Hite 3350 filter, with a bandpass between 9-11 Hz, so as to eliminate other frequencies that are not associated with startle. This filter's output was fed into two channels in a Dynograph R411 pen recorder (Beckman Instruments)

Fig. 1 Photograph of the startle box, showing the
accelerometer resting on the adjustable pedestal.



and also simultaneously fed into a model 511 dual beam Tektronix storage oscilloscope. Before the start of each experimental day, the filter's output was fed into a Hewlett-Packard (HP) 5300A frequency measuring system and HP5304A Time/Counter, to check the accelerometer output, while adjusting the springs for optimal frequency output. This was done in order to calibrate the accelerometer, and hence have a standard waveform output from the the cage before tests, across all the experiments to be conducted.

An Exidy Sorcerer computer with 8-bit A/D and D/A converters controlled the stimuli and collected the data. The output of a solid state relay switch was fed into a model 829E Grason-Stadler electronic switch, that controlled a model 455C Grason-Stadler noise generator (Grason-Stadler, West Concord, Mass.). The signal was then amplified by a Sony TA-3650 amplifier (Sony Corp.). The output of this amplifier was then fed into a loudspeaker mounted in the test chamber. This output constituted the test stimuli, the background noise being taken from another channel in the noise generator, through the electronic switch and amplifier, and then into a tweeter also mounted in the test chamber.. The pen recorder's outputs were then fed into the computer's A/D converter. The A/D converts the pen movements to between 0-255 units.

In order to capture a wide range of response

amplitudes, the startle output from the cage was fed into two channels in the pen recorder that were designated as high (50 mV/cm) and low (200 mV/cm) sensitivity channels, so that a wide range of response could be captured when necessary. These two settings had been found from previous work in this laboratory, to be sensitive enough to record the "high and low startling" rats. The outputs from the recorder were also fed into two separate ports in the A/D converter, so that when the 50 mV/cm pen reaches 255, which was the maximum pen deflection, the 200 mV/cm pen was still able to record that startle since, it's 4 times less sensitive than the 50 mV/cm pen.

CALIBRATION:

A typical baseline and test day began by checking the intensity levels of the 20 msec 110 db stimulus and the 70 db background noise with a noise/sound level meter and adjusting accordingly, if necessary. The accelerometer's output was checked by placing a 12 cm woofer (Radio Shack) with the cone and center piece cut out, into the startle cage, and then placing a total of 300 gm lead weights on it. The woofer was then vibrated with a 10 Hz sine waveform from a HP 3311A function generator, to simulate the startle frequency, which has been found to occur around this frequency (Davis and Cassella, 1984 Personal Communications). The accelerometer's output was then

monitored on the oscilloscope while the tensions in the startle cage springs were being adjusted by means of the pedestal mentioned above, until a perfect 10 Hz sine wave could be seen on the oscilloscope and at the same time traced out by the pen recorder. When this had been achieved, it meant that startle cage resonance had virtually been eliminated. The lead weights and woofer were then removed and a dry run of the whole set up was then made to set the recording pen's level.

TESTING:

On each test day, which was always the day after baseline measurement, rats were injected with the drugs or saline according to which group they belonged. Using a spring covered polyethylene tubing with internal and external diameters of 0.5 mm and 1.56 mm respectively (Plastic Products), a 27-gauge internal cannula cut so that only about 0.5 mm tip protruded when inserted into a 6.5 mm guide cannula. This internal cannula was fitted into one end of the tubing, while a 1 cc injection syringe was inserted at the other end. Distilled water was then slowly drawn into the tube via the internal cannula until the whole length of the tubing was filled, taking care that no air bubble was let in. An extra 0.8 cc of water was also drawn into the syringe so that about 0.4 cc jet of water could be pushed out, to be

absolutely sure that there was no air bubble in the tubing. A pair of haemostats was then used quickly to clamp the tubing very close to the syringe and the syringe was then removed.

A 5 μ l microsyringe (Hamilton, Reno, Nevada) was placed in an injection pump with a variable injection duration, but set at an injection duration of 10secs. to coincide with the interstimulus interval (ISI) used in this study. Part of the tubing behind the haemostat was cut leaving about 5 mm length, into which the microsyringe was inserted and then the haemostat was removed. The tube was then inserted, internal cannula end first, through a hole on the Lafayette box, into the startle cage. A 0.5 μ l air pocket was drawn into the tubing and 4 μ l of the drug or saline to be injected was also immediately drawn in. The injection pump and the free flow of fluid was then tested by using the pump to eject 1 μ l of the substance to be injected.

Each rat was then brought out of the holding room, the dummy cannula was unscrewed from the rat's head and another internal cannula cut to the same length as the one injector cannula, was inserted twice into the guide cannula and withdrawn to clear the immediate surrounding of the cannula tip of blockage if there happened to be any. The injector cannula at the end of the tubing was then inserted into the guide cannula. The rat was then

put in the startle cage and the sound attenuating test chamber door shut.

The rat was given 5 mins to readjust to the startle cage. At 20 secs before the end of this 5-min. period, the background noise was turned on. At the termination of this 20-sec period, the computer was then used to start the stimulus. The stimulus continued to be delivered until at exactly after the 20th trial, when the injection pump was started to administer the desired volume of drug or saline over the 10-sec ISI period, after which it was turned off. The startle programme continued to run until the end of each session which was the 80th trial. The pen was then switched off, and the rat removed.

Histology:

A volume of dye (Carter's drawing ink) equalling that amount used in the experiment, was injected into the MSB and the rats sacrificed 10 mins later. The brains were removed, and frozen fresh in a cryostat for 1hr at -7degrees celsius. On the same day, the brains were sliced until the beginning of the MSB could be seen, wherein every third slice at 40 μ m thickness was mounted on a glass slide. The mounted brain sections were then stained with cresyl violet, for viewing under a projection microscope. After each run in the EEG experiments (see Appendix), a Grass LM radio frequency lesion maker (Grass

Medical Instruments, Quincy, Mass.) was used to make a lesion at the site of the recording electrode tip by passing a 10 mA current for 40 secs; dye was then injected into the MSB, and the same protocol was followed as above, except that after the MSB, slicing continued without collecting any of them, until the first indication of the lesion was observable. At this point, every second slice at 40 μ m thickness was taken and also mounted on a glass slide. The same protocol for staining described above was followed.

GLUTAMATE INJECTIONS

Intraseptal injections of L-glutamate (GLUT) should decrease the startle amplitude by exciting the neurons in the MSB.

Experiment I:

Method: Nine days after surgery, each rat was habituated once for 10 mins, to the test chamber. This habituation period used neither the stimulus nor the background noise, it was only meant to accustom the rat to the test box. The test chamber was illuminated by two-24V D.C. light bulbs which were kept on at all times during the habituation and test periods.

The next day, each animal was placed in the test chamber and allowed 5 mins to readapt to the box without any stimulus. After this period, a 70 db background noise and 80 trials of the test stimulus (110 db, 20 msec duration, and 10 sec ISI) were given and the mean of the startle amplitude was then used to divide the rats into the different groups required. Group allocation was done by rank ordering the means for all the subjects, and then dividing them into groups so that a 1-way ANOVA performed

on the means yielded no significant difference between the groups(e.g., for experiment III, $F(2,23) = 0.11$, $p > 0.45$).

Thirty rats were divided into three groups using the baseline procedure detailed above. The groups were assigned the following treatments: 0.5 M (GLUT), 1 M GABA and 0.9% w/v Saline(SAL), with an injection volume of 1.5 ul. Testing was performed the next day after baseline as described earlier in the text, and the data used in the analysis were the 21st-25th trials of the baseline day and the 21st-25th trials of the test day.

Results: In analysing the data from this study, the baseline used was measured a day before the test. Data from this test were converted to percentage of baseline and a one-way between subjects ANOVA test was performed on the converted data.

Table 1 is the summary table of the ANOVA, which indicated no significant difference across the groups ($F(2,27) = 1.28$, $p > .20$). This non significance is shown in fig.2 of the plot of the group means ($M_{GLUT} = 92.3$, $M_{GABA} = 107.2$ and $M_{SAL} = 99.1$). Table 2 shows the result of another ANOVA performed on the data, but this time using the 16th-20th trials of the test day as baseline. This analysis also indicated a no significant difference across the groups ($F(2,27) = 2.30$, $p > .10$), which is also demonstrated in fig.3 with group means as

follows: $M_{GLUT} = 120.1$, $M_{GABA} = 221.14$, and $M_{SAL} = 150.37$.

Conclusion: The results of this experiment show that there were tremendous variations between and within the subjects. This was particularly demonstrated in the differences between the MSe of the two analyses. When a separate day baseline was used, the MSe for that analysis was 20948.67, as compared to MSe of 2180.23 when the 16th-20th trials of the test day was used as baseline.

Other contributions to the differences in variations could have come from the mode of injection and the variation in speed of injections between subjects.

Experiment II:

The baseline used in the previous experiment was measured a day before the test and it was later found to be mostly unreliable because there were tremendous variations in the startle amplitudes between the baseline and test days. These variations were interpreted to have been due firstly, to the interval separating the baseline and the test periods. Secondly, because the injection syringe did not utilize a pump for experiment I, there were

TABLE 1

Experiment I: Startle amplitude analysis of
1.5 μ l injections in the .5 M GLUT,
1 M GABA, and SAL. groups (using the
21st-25th trials from baseline measured
a day previous to the test day as
the baseline).

| Source | SS | df | MS | F |
|------------|-----------|----|----------|------|
| Treatments | 53786.1 | 2 | 26893.05 | 1.28 |
| Error | 565614.04 | 27 | 20948.67 | |

Fig. 2 Means of the startle amplitudes of rats in experiment I expressed as a percentage of the mean of the 41st-25th trials of baseline, measured a day previous to the test day (n=10).

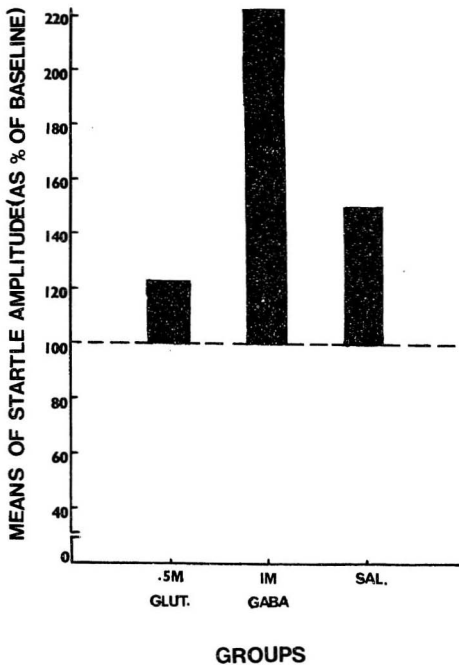


TABLE 2

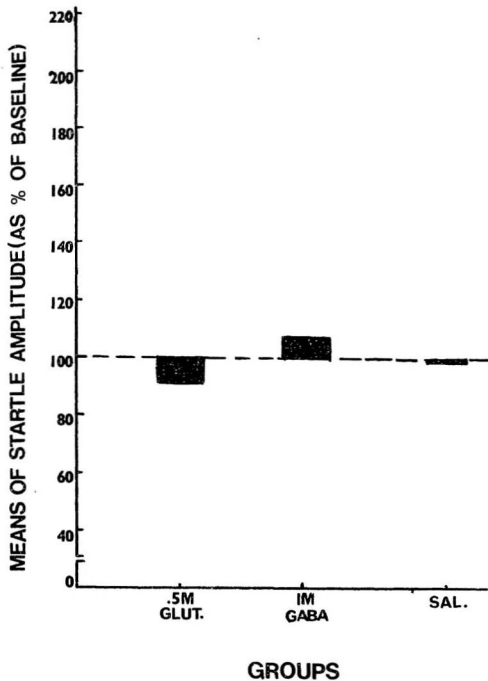
Experiment I: Startle amplitude analysis of
1.5 μ l injections in the .5 M GLUT,
1 M GABA, and SAL. groups (using
the 16th-20th trials of the test day
as the baseline).

| Source | SS | df | MS | F |
|------------|----------|----|---------|------|
| Treatments | 10007.91 | 2 | 5003.95 | 2.30 |
| Error | 58866.3 | 27 | 2180.23 | |

Fig. 3 Means of the startle amplitudes of rats in experiment I expressed as a percentage of the mean of the 16th-20th trials from the test day ($n=10$).

1.2

100



bound to be differences in duration of injection and the amount of pressure applied.

Because of the very high variations in the startle amplitude between the baseline and test days, subsequent experiments used a same day baseline for analysis. This meant that the baseline measured a day before testing was henceforth only used to divide the subjects into groups.

Method: This experiment had 22 rats that were divided into two groups and then assigned the following treatments: .5M GLUT and SAL

Results: On the test day, the data used for baseline calculations were means of the 5 (16th to 20th) trials immediately before infusion and because neither the effects of GABA nor GLUT lasts very long in the MSB, only the means of the 5 (21st to 25th) trials immediately after the injection was used as the test measurement.

Table 3 is the summary of the one-way ANOVA F-test performed on the results after being converted to percentage of the baseline, while fig.4 shows the groups' means ($M_{GLUT} = 77.7$, and $M_{SAL} = 91.46$) as a percentage of the baseline. The result of the analysis showed no significant difference ($F(1,20)=1.05$, $p > .20$) between the L-glutamate and GABA subjects in expt.II.

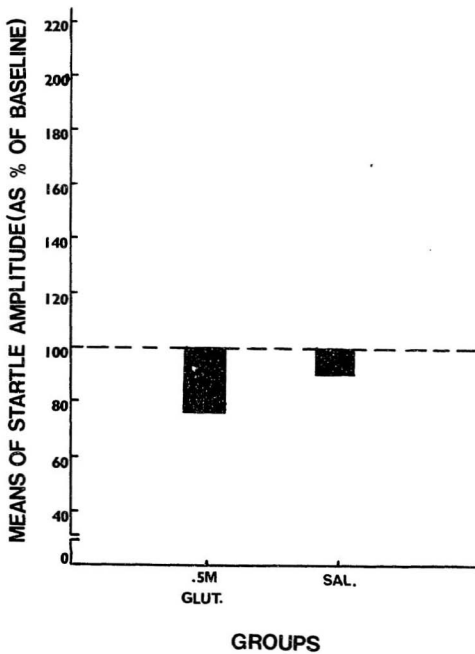
Conclusion: Though this experiment showed no

TABLE 3

Experiment II: Startle amplitude analysis of
1.5 μ l injections in the .5 M GLUT,
and SAL. groups.

| Source | SS | df | MS | F |
|------------|---------|----|---------|------|
| Treatments | 1071 | 1 | 1071 | 1.05 |
| Error | 20476.2 | 20 | 1023.81 | |

Fig. 4 Means of the startle amplitudes of rats in experiment II, expressed as a percentage of test-day baseline (n=11).



significant difference between the groups, the MSe in this experiment was considerably reduced, as compared to that in experiment I (Expt. I MSe = 2180.23 vs. Expt. II MSe = 1023.81). This suggested that the use of an injection pump in this experiment resulted in a considerable reduction in the variations within subjects.

Experiment III:

For this experiment, the volume injected was decreased from 1.5 μ l to 1 μ l, and the GLUT concentration increased from .5 M to 1 M. Changes were made because it was thought that the volume injected in the last two experiments could have contributed to the within group variation seen in those experiments, by exerting pressure during injections, on the MSB cells. It was also thought that the ineffectiveness of GLUT might have been due to the fact that the concentration used in the last two experiments was too low.

Method: Experiment III had 30 rats that were divided into three groups as follows: .5 M GLUT, 1 M GLUT, and SAL, and then given 1 μ l injections.

Results: Three rats lost their guide cannula when the dental cement came off in their home cages while the placement in a one was outside the MSB. Consequently only the data from 27 rats were used in this analysis, nine each in the .5, and 1 M L-GLUT groups and eight in the

saline group. An unweighted means, one-way ANOVA was performed and the result is shown in table 4. The summary table shows a significant difference ($F(2,23) = 4.31, p < .05$) among the three groups. A Newman-Keuls analysis performed on the differences among the group means ($M_{.5M\text{ GLUT}} = 89.25, M_{1M\text{ GLUT}} = 51.16, \text{ and } M_{SAL} = 103.26$), showed that there were significant differences between the .5, and 1 M GLUT groups and also the 1 M GLUT and SAL groups, but not between the .5 M GLUT and the saline groups (fig.5 and table 5).

Conclusion: The results of these experiment show that the volume and concentration used in expts. I and II might have contributed to the ineffectiveness of GLUT seen in those experiments. It can then be concluded that intraseptal injection of 1 μ l, 1M GLUT significantly attenuated the rat's ASR.

TABLE 4

Experiment III: Startle amplitude analysis of
1 μ l injections in the .5,
1 M GLUT, and SAL. groups

| Source | SS | df | MS | F |
|------------|----------|----|---------|-------|
| Treatments | 12561.23 | 2 | 6280.61 | 4.31* |
| Error | 33473.33 | 23 | 1455.36 | |

* $p < .05$

Fig. 5 Means of the startle amplitudes of rats in experiment III, expressed as a percentage of test-day baseline (n=9 for .5 M, and 1 M GLUT. groups, n=8 for SAL. group).

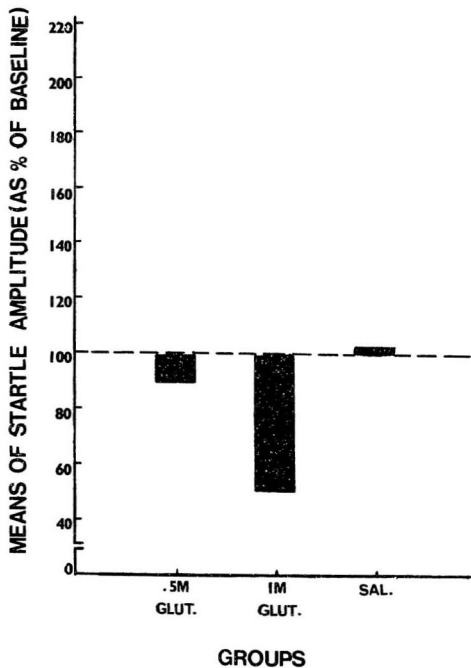


TABLE 5

Newman-Keuls analysis of the means from groups in experiment III.

| | II (1M GLUT.) | I (.5M GLUT.) | III (SAL.) |
|-----------|---------------|---------------|------------|
| \bar{X} | 51.16 | 82.95 | 103.26 |
| II | - | 38.09* | 52.01* |
| I | - | - | 14.01 |
| III | - | - | - |

* $P < .05$

Experiment IV:

Method: Because it was thought that 1 μ l was still a relatively large volume to inject into the MSB, a smaller volume was tried to see if the same effect observed for the 1 μ l injection could also be seen here. In order to test whether this was a correct reasoning, experiment IV had 27 rats divided into three groups, and assigned treatments as follows: .5 M GLUT, 1 M GLUT, and SAL, but this time injecting .5 μ l instead of 1 μ l, as was done in expt. III.

Result: In this study, a total of six rats had to be rejected because four of them lost their guide cannulae in their home cages, while the other two were found to have cannula placements outside the MSB. Figure 6 shows the mean response amplitudes for each group ($M_{\text{GLUT}} = 130.27$, $M_{\text{1M GLUT}} = 180.44$, $M_{\text{SAL}} = 128.81$). Subsequent analysis of this data revealed no significant differences ($F(2,18) = 0.68$, $p > .05$), as depicted in table 6.

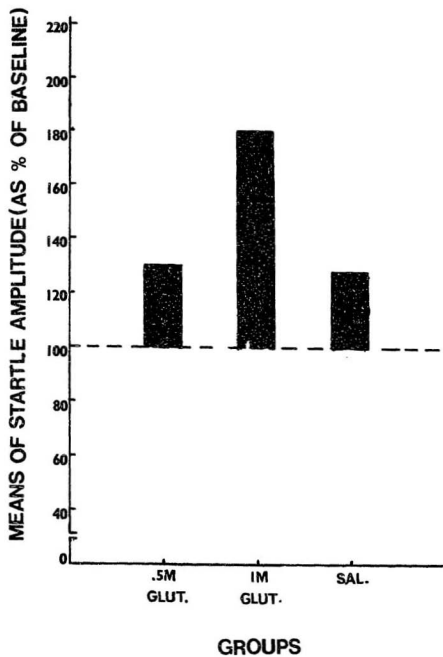
Conclusion: It may be concluded that decreasing the volume injected from 1 μ l to 0.5 μ l resulted in the effects observed in expt.III being eliminated in expt.IV.

TABLE 6

Experiment IV: Startle amplitude analysis of .5 μ l injections in the .5, 1 M GLUT. and SAL. groups.

| Source | SS | df | MS | F |
|------------|-----------|----|---------|-----|
| Treatments | 12097.98 | 2 | 6048.94 | < 1 |
| Error | 159460.18 | 18 | 8858.90 | |

Fig. 6 Means of the startle amplitudes of rats in experiment
IV, expressed as a percentage of test-day baseline (n=7).



GABA INJECTIONS

In this section, intraseptal injection of GABA should significantly elevate startle amplitude by inhibiting neurons in the MSB.

Experiment V:

Method: 30 rats were divided into three groups and assigned treatments as follows: 1 M GABA, 1.5 M GABA, and SAL., given in 1 μ l injections.

Results: Table 7 shows the results of the analysis of the data from expt.V, treated as a percentage of the baseline. There was no significant difference ($F(2,27) = < 1$, $p > .05$) between the group means ($M_{1M\text{ GABA}} = 122.3$, $M_{1.5M\text{ GABA}} = 119.65$, and $M_{\text{SAL}} = 119.64$), shown in fig.7.

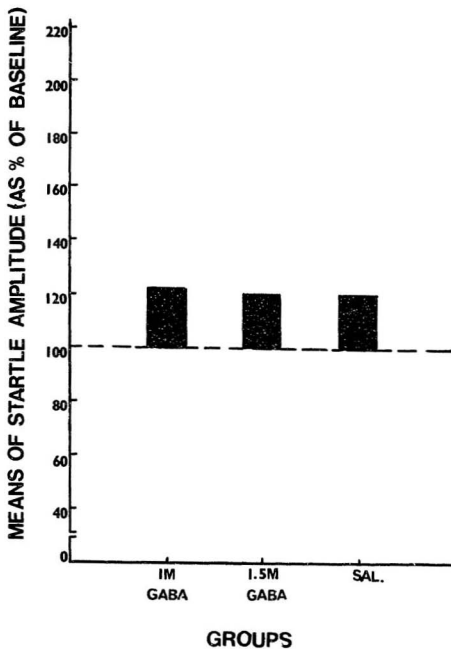
Conclusion: The results of this experiment show that the concentrations and volumes of GABA used had no effect in modulating the startle reflex, and this might have been due to the high variations within subjects, as indicated by the MSe, which was 21737.61.

TABLE 7

Experiment V: Startle amplitude analysis of 1 μ l
injections in the 1, 1.5 M GABA
and SAL. groups

| Source | SS | df | MS | F |
|------------|-----------|----|----------|-----|
| Treatments | 0.4825 | 2 | 0.241 | < 1 |
| Error | 586915.35 | 27 | 21737.61 | |

Fig. 7 Means of the startle amplitudes of rats in experiment
V, expressed as a percentage of first-day baseline (n=10).



Experiment VI:

Method: This experiment sought to decrease the variations mentioned above by decreasing the volume and increasing concentration of the injectate. To this end, 27 rats were divided into three groups and assigned treatments as follows: 1.5 M GABA, 2 M GABA, and SAL, and given .5 μ l injections instead of 1 μ l injections, as was done in expt. V.

Results: Table 8 is the result of the ANOVA performed on the converted data. There was no significant difference among the group means ($M_{1.5M \text{ GABA}} = 99.6$, $M_{2M \text{ GABA}} = 102.1$, and $M_{SAL} = 105.8$), shown in fig 8.

Conclusion: The results of the GABA experiments suggest that GABA had no effect in modulating the rat's startle amplitude.

TABLE 8

Experiment VI: Startle amplitude analysis of .5 μ l
injections in the 1.5, 2 M GABA and
SAL. groups

| Source | SS | df | MS | F |
|------------|----------|----|---------|-----|
| Treatments | 177.33 | 2 | 88.66 | < 1 |
| Error | 48633.77 | 24 | 2026.41 | |

Fig. 8 Means of the startle amplitudes of rats in experiment VI, expressed as a percentage of test-day baseline (n=9).

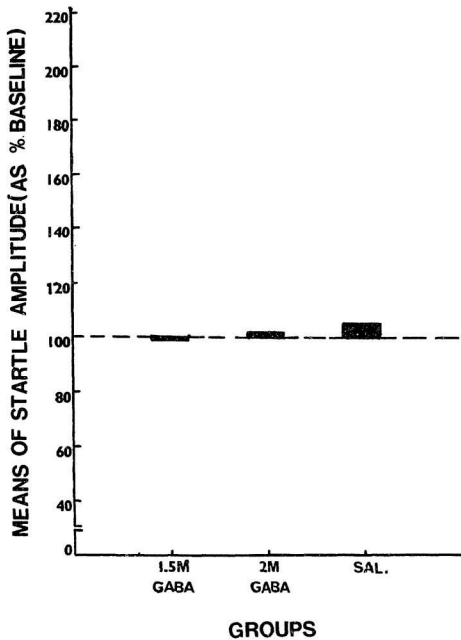
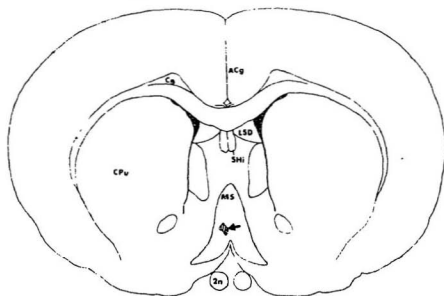
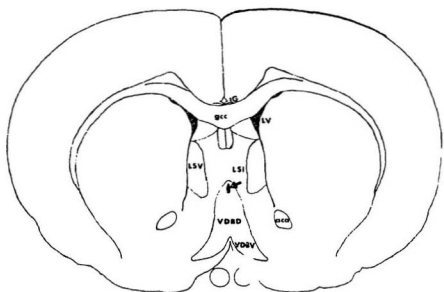


Fig. 9 Schematic diagrams of internal cannula tip sites of rats used in all the analyses. The top and bottom arrows show the most dorsal and ventral penetrations of the internal cannulae, respectively.

Legend: aca- Accumbens nucleus, ACg- Anterior cingulate cortex, Cg- Cingulum, CPu- Caudate Putamen, gcc- Genu corpus callosum, IG- Induseum griseum, LSD- Lateral septal nucleus, dorsal., LSI- Lateral septal nucleus, inter., LSV- Lateral septal nucleus, ventral., LV- Lateral ventricle, MS- Medial septal nucleus, SHi- Septohippocampal formation, VDBD- Nu. vertical limb diagonal band, dorsalis., VDBV- Nu. vertical limb diagonal band, ventralis, 2n- Optic nerve (Legends after Paxinos and Watson, 1982).



Bregma 0.7mm

DISCUSSION

Davis has proposed that the rat's ASR is mediated by a brainstem neural circuit and therefore all other pathways that modulate the ASR have been viewed as impinging on this neural circuit. The removal of the MSB and hence its efferent connections has led to an impairment of habituation of the startle response. By specifically exciting or inhibiting neuronal cell bodies in the MSB, the present study has attempted to investigate the involvement of this area in the modulation of the rat's ASR.

GLUTAMATE:

Though injections of L-GLUT in experiments I and II did not yield any significant differences, they however, served to eliminate some variables that could have confounded the finding in experiment III. The result of experiment IV may be taken as indicating the optimum volume for injection of L-GLUT into the MSB, in order to achieve modulation on the rat's startle amplitude.

As a result of the finding in experiment III, it has been concluded that intraseptal injection of L-GLUT resulted in attenuation of the rat's startle amplitude.

This was presumably achieved by increasing the firing rate of the SH neurons and thereby increasing the release of transmitters into the hippocampus. Since Goodchild, Dampney and Bandler (1982) have shown that L-GLUT excites only cell bodies and not axons of passage, it may then be suggested that in this study, the cell bodies in the MSB were probably responsible for attenuating the rat's startle amplitude.

Specifically how glutamate achieves this startle amplitude attenuation is still speculation. The MSB has been shown to project not only to the hippocampus, but also to the amygdala (Price, 1981) and the hypothalamus, and since L-GLUT excited all cell bodies in the MSB, including the ones that project to both the amygdala and the hypothalamus, the involvement of these other projections in startle amplitude modulation cannot be ruled out. In essence, inferences drawn from these glutamate studies are limited to its action in the MSB alone.

GABA:

Injectons of different concentrations and volumes of GABA into the MSB did not alter the rat's acoustic startle amplitude. The very low experimental groups' mean square value obtained in the analysis of data from the two experiments; suggests high levels of variations among the

subjects within groups on test days. GABA has been shown to be a potent inhibitor of the MSB neurons (Segal, 1974), causing a cessation in the spontaneous firing rate of these cells. With cessation of the spontaneous firing rate of the MSB cells, a decrease or cessation of ACh release in the hippocampus should be expected.

The close metabolic relationship between GABA and glutamate introduces the possibility that some of the GABA injected in the present study might have been converted to glutamate, hence decreasing the amount of GABA available to the MSB neurons. The conversion of GABA to glutamate was reported by Vellucci and Webster (1985) to have had an effect on the efflux of [14 C]-label from rat spinal cord slices pre-incubated with [14 C]L-glutamic acid, when GABA was applied, but after adding amino-oxyacetic acid (AOAA), a GABA transaminase inhibitor, to the mixture, an inhibition of [14 C]-label efflux was reported. So in this experiment, the conversion of part of the injected GABA to glutamate might have nullified the effect of the remaining GABA, hence making the effect equal to the injection of saline.

Apart from being converted to glutamate, some of the the GABA might also have been taken up by presynaptic terminals in the vicinity of the injection site, but this calls for GABAergic terminals to be present in the MSB. Costa et al. (1983) have reported that the MSB releases

GABA, suggesting that there are terminal boutons in this area that sequester and release this transmitter. Moreover, Allen and Crawford (1985) injected muscimol into the MS of rats, to study its effects on the hippocampus and ACh utilization. ACh utilization was reported to have been reduced while the hippocampal electrographic recordings showed low amplitude asynchronous waves and less theta wave activity. Since MS neurons were affected by localized injections, they suggested that the pacemaker-driving function of the MSB neurons is subject to GABAergic actions. The findings of these two studies put together, not only indicated the existence of GABAergic terminals, but that the released GABA affected the activities of the SH neurons in the MSB. In the light of these findings, it may be suggested that some of the GABA injected in the present study might have been inactivated by uptake into GABAergic terminals in the MSB.

Another possible explanation for the ineffectiveness of GABA in the present study could have been that of floor effect. That is, the MSB cells were already firing at their resting level, and GABA was unable to depress them further. It should be noted however, that this reasoning is in contradiction of the Segal(1974) finding. On the other hand, L-GLUT. manifested its effect by exciting these cells.

Suggestions for further experiments:

Experiments could be performed that would include inhibiting the uptake of GABA and its conversion to glutamate. The use of AOAA, as was done in the Vellucci and Webster (1985) study, to block the conversion of GABA to glutamate, might ensure a longer presence of the injected GABA. If beta-Alanine, a GABA uptake inhibitor is administered with AOAA, then GABA would not only be protected from being converted to glutamate, its uptake into presynaptic terminals would also be impaired resulting in a double assurance that the injected GABA will remain available to the MSB neurons until its been taken up by the presynaptic cells.

It may be possible to replicate the L-GLUT. effect in the present study by substituting GABA antagonists like bicuculline or 5-aminovaleric acid (Krogsgaard-Larsen, 1985), for L-GLUT. Agardh (1986) has shown that GABA antagonists like bicuculline and picrotoxin were able to increase the release of [^3H] ACh from the chick retina. This increase in ACh release from the chick retina is similar to the effect that the intraseptal injection of L-GLUT. might have had on the turnover rate of hippocampal ACh, in the present study. If the glutamate experiment in the present study is repeated using a GABA antagonist instead of L-GLUT., and the same result is still seen, this may buttress the argument that the effect

of L-GLUT. reported in the present study was due to its ability to increase ACh release in the rat's hippocampus.

Another question which needs to be answered is the identity of the transmitter(s) in the MSB involved in modulating the rat's ASR. Costa et al., (1983) reported the presence of cell bodies and terminals containing different putative transmitters. For instance, Blaker et al., (1984) reported that intraseptal injection of SP decreased ACh turnover rate in the rat's hippocampus. Lamour et al., (1984) have also reported that microiontophoresed SP had a strong excitatory influence on MSB neurons. These two studies together suggest that for SP to be an excitant in the MSB, and at the same time produce a decreased ACh turnover rate in the hippocampus, there are probably SP terminals in the MSB which synapse on the inhibitory interneurons, which in turn affect the activities of the cholinergic SH neurons. If a SP antagonist like DPDT-SP (Hokfelt, Vincent, Rosell, Folkers, Markey and Cuello, 1981) or Spantide-SP is injected into the MSB, the ACh turnover rate should be increased and thereby decrease the startle amplitude. In effect, using a SP antagonist should produce the same behavioural result as GABA was supposed to, if SP plays a role in the mediation of the rat's startle amplitude.

NE is another transmitter reported to be present in the MSB. Segal (1974) reported that intraseptally

injected NE inhibited the spontaneous firing rate of MSB neurons, which suggests a likelihood of a decrease in ACh turnover rate in the hippocampus, and this should result in an increase in startle amplitude. If centrally applied NE inhibits spontaneous firing in the MSB, then injecting a drug like clonidine, with NE antagonistic properties, should increase ACh turnover rate in the hippocampus and thereby depress startle amplitude. This method of challenge using agonists and antagonists to the different putative transmitters may be useful in determining which of the transmitters in the MSB have direct effects on SH neurons, and which ones recruit interneurons in order to achieve the effects attributed to them.

In summary, this study has shown that injections of L-glutamate into the MSB attenuate the rat's ASR. In essence, this study has been able to confirm part of the suggestion of Miller and Treft (1979) that the MSB (in this case, MSB cell bodies) are involved in modulating the rat's ASR. Hippocampal EEG recordings have shown that the duration, volumes, and concentrations of injections used in this study did not induce any epileptiform-like activity in the hippocampal CA1 area (see the appendix for more details).

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APPENDIX A

Effects of septal injections of glutamate, GABA,
and picrotoxin on Hippocampal EEG.

The next set of experiments were performed to see if the volumes and concentrations of the drug used in all the above experiments were resulting in epileptiform-like activity in the hippocampus, the projection target of the septo-hippocampal fibres. The rationale was that, if there was epileptiform-like activity, then the effects of the drugs found in the glutamate and GABA experiments would have to be attributed to their capacity to induce abnormal activity, rather than normal pharmacological effect.

To this end, EEG recordings were taken from anaesthetised and awake animals, during intraseptal injections with the drug or saline. For the acute recordings, 10 rats were anaesthetised with Urethane (.75 cc/100 g body wt.) and a guide cannula implanted as described above. Two male amphenol plugs with teflon coated, tungsten wire of 0.0762 mm diameter soldered in place, were used as electrodes. The recording electrode was a 5 mm long tungsten wire with only the tip exposed, soldered to an amphenol plug. This was lowered into the dorsal hippocampus at the CA1 level, using the following

coordinates: -3.3 mm bregma, 4.0 mm lateral, and 3 mm ventral from the skull surface. The negative electrode was a 10 mm bare tungsten wire wound around one of the skull screws.

All concentrations and volumes of the two drugs, that had been used in the previous experiments, and saline used were tested for their effects on the hippocampal EEG. Each rat served as its own control, that is, saline was always injected to make sure that the mechanical distortion on the cells of the MSB caused by the injection did not have any noticeable effects on the EEG. The pen recorder was allowed to run for 1 min before injection, during the injection, which was 10 sec, and 3 mins after the end of injection.

For the recordings in the awake animals, 10 rats were anaesthetised with Sodium Pentobarbital (Somnotol) injections, and guide cannulae implanted into the MS according to the earlier protocol. The positive and negative electrodes were implanted in the same way as they were implanted for the recordings in anaesthetised animals, but this time, a ground electrode was made by soldering a 30 mm bare tungsten wire to a male amphenol plug. The base of the ground electrode's amphenol plug was placed on the skull, just caudal to lambda, the tungsten wire was bent and embedded subcutaneously along the neck region. All three electrodes were then held in

place by dental cement. The rats were allowed 8 days to recover before testing.

All concentrations of GABA and L-GLUT. used in this study were tested by intraseptal injections, and EEG recordings made in the same manner as was done for the anaesthetised animals.

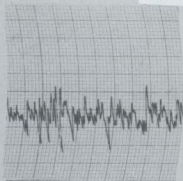
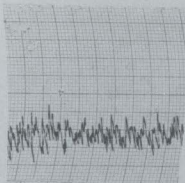
RESULTS AND CONCLUSIONS

No tests conducted on anaesthetised subjects revealed significant effects on the EEG patterns.

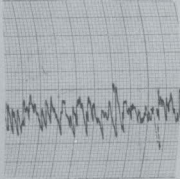
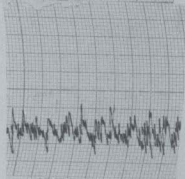
As it was for the anaesthetised subjects, there was no noticeable change or effects of injecting 0.5 μ l, or 1 μ l, 0.9% w/v saline, on the hippocampal CA1 EEG of the awake subjects (left hand panels of figs. A1 and A2). Also like the anaesthetized subjects, neither 0.5 M, nor 1 M L-GLUT. had any effect on the EEG patterns of the awake subjects (Figs. A1 and A2). The left and right panels of figs. A3 and A4 showed no effects of 0.5 μ l injections of 2 M and 4 M L-GLUT. on the EEG patterns. Only two concentrations (1 M and 2 M) and one volume, 0.5 μ l, of GABA were tested on awake subjects. Just like the glutamate subjects, GABA injections revealed no effects on the EEG patterns, at any point during its measurement.

Fig. A1 EEG recordings from awake animals. Unless otherwise indicated, recordings in this appendix were taken at 1 sec before (A), during (B) injection, 10 sec (C), and 40 sec (D), after injection. The left and right hand panels show recordings from .5 μ l SAL., and .5 μ l .5 M GLUT. rats, respectively.

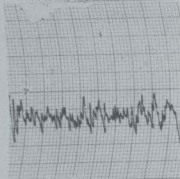
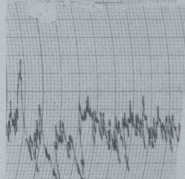
A



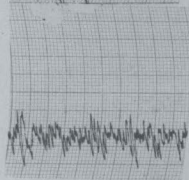
B

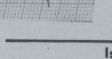


C



D



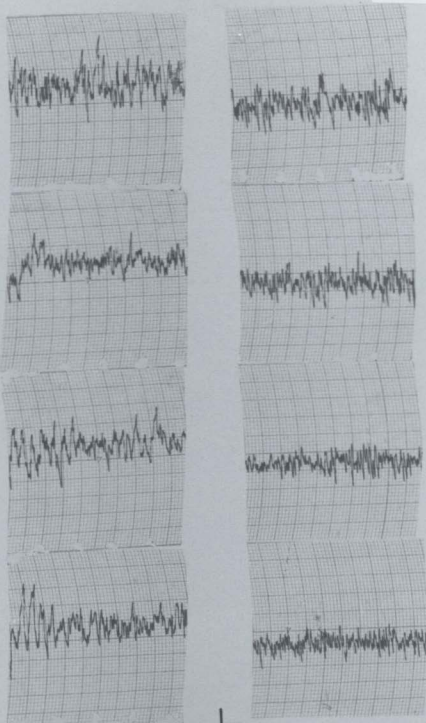

 .5mV
 Is

A

B

C

D



Electrophysiological traces (A, B, C, D) recorded on grid paper. The traces show high-frequency, irregular oscillations. A scale bar indicates 1s (horizontal) and .5mV (vertical).

1s

.5mV

Fig. A2 The left hand panel shows recordings from 1 μ l SAL.
rat, while the right hand panel shows recordings from a 1
 μ l, 1 M GLUT. rat.

Fig. A3 The left and right hand panels show recordings from .5 μ l, 2 M GLUT., and .5 μ l, 1 M GABA rats, respectively

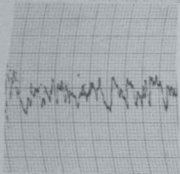
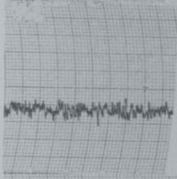
A



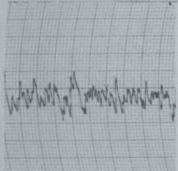
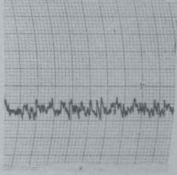
B



C



D

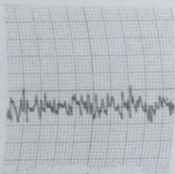
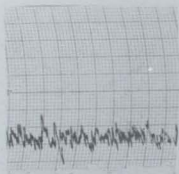


.5 mV
1s

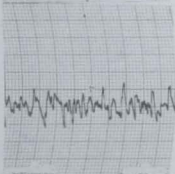
.5 mV
0.4s

Fig. A4 The left and right hand panels show recordings from .5 μ l, 2 M GABA, and .5 μ l, 4 M GLUT. rats, respectively.

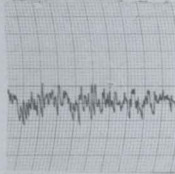
A



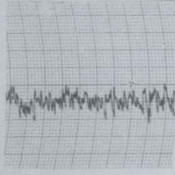
B



C



D



.5 mV
1s

Effects of Picrotoxin:

Picrotoxin was used in the present study to demonstrate that the effect of pharmacologically manipulating the MSB could be recorded from the hippocampus. This was thought necessary because of the lack of effect seen with 4 M L-GLUT. injections, on the EEG. It could be argued that the reason why no effect was seen with L-GLUT. and GABA injections might have been because pharmacological manipulations of the MSB could not be seen in the types of records that were being made. If epileptiform-like activities could be recorded in the hippocampus after injecting picrotoxin into the MSB, then it can be clearly stated that the effect of pharmacological manipulations of the MSB can be monitored in the hippocampus.

Fig. A5 shows the EEG patterns from subjects injected with 1 μ l saline. These two panels are for the controls for the two concentrations (4, and 6 mg/ml) of picrotoxin used. As expected, saline injections did not have any effects on the EEG. Injection of 1 μ l, 4 mg/ml picrotoxin resulted in hyperkinesis, gnawing, and sometimes crawling on the stomach. about 21 mins after injection, vibration of the head was noticed, accompanied by chewing and ear wiggling, but there was no epileptiform-like activity in the EEG.

Figure A6 are samples of the EEG patterns from the 1

1 μ l, 6 mg/ml picrotoxin subject. In this subject, hyperkinesis, as described for the 4 mg/ml subject, was noticed 3 min after injection, but hyperkinesis in this subject included walking in circles or sometimes walking backwards. At 6.5 mins after injection, interictal spikes were noticed and this behaviourally corresponded to the subject supporting itself on three paws (left front paw lifted off the ground) for about 1 min. Hyperkinesis continued until about 11 mins after injection when the subject started rearing on its hind legs, chewing and shaking the front paws. Epileptiform-like discharges were noticed at 10 mins after injection (fig. A6). The rearing and ear wiggling continued and at one point, a behaviour similar to the "wet dog shake" was observed. The EEG panel, showing epileptiform-like bursts at about 18 mins, indicates how frequent the bursts were. These continued for another 20 mins, after which the rat was anaesthetised.

In the present study, 1 μ l injections of picrotoxin were used not only because it was the volume that produced a significant effect in the L-GLUT. study, but also because 1 μ l, 2 mg/ml in artificial CSF had been injected by Davidson and Barbeau (1975), into the rat's fastigial nucleus, and was reported to result in minor epileptic behaviors. Higher doses were chosen in this study with the hope that epileptiform-like activities could be

recorded from the hippocampus after septal injections of picrotoxin. The hyperkinesis and epileptiform-like discharges shown at 6.5 mins and 10 mins respectively, by the 6 mg/ml subject, is in line with the finding that ventricular (lateral) injections of picrotoxin (3 to 30 $\mu\text{g/kg}$) in dogs, was reported to have resulted in convulsive discharges, with onset between 10-12 min and lasting 75-100 mins (Bircher, Kanai, and Wang, 1962).

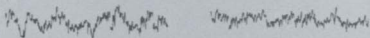
Since the picrotoxin induced epileptiform-like activity in the hippocampal EEG was observable in the recordings, the lack of interictal or epileptiform-like discharges in the GABA and GLUT. experiments was not as a result of methodological failure because they can be shown in the picrotoxin experiment. Therefore, it is unlikely that the volumes and concentrations of injectates used in this study had their effects on the startle amplitudes by altering EEG wave forms to the extent of producing the interictal or epileptiform-like discharges in the hippocampal CA1.

Fig. A5 EEG recordings from picrotoxin animals. The left and right hand panels show recordings from 1 μ l saline control animals for 4 mg/ml and 6 mg/ml picrotoxin injections respectively.

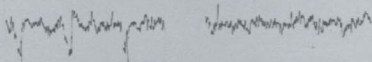
A



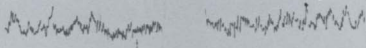
B



C



D



.2 mV
Is

.5 mV
Is

Fig. A6 Panels A to I show recordings from the 6 mg/ml
picrotoxin rat. The recordings were taken at 1 sec before
(A), during (B) injection, 10 sec (C), 40 sec (D), 3 min
(E), 4.5 min (F), 6.5 min (G), 10 min (H), and 18 min (I)
after injection, respectively.

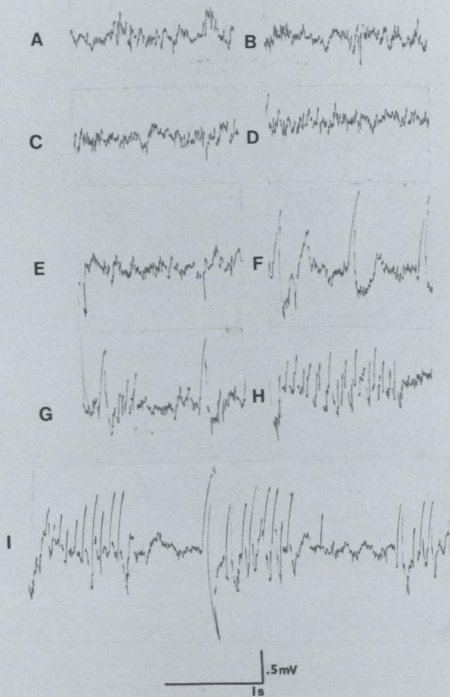
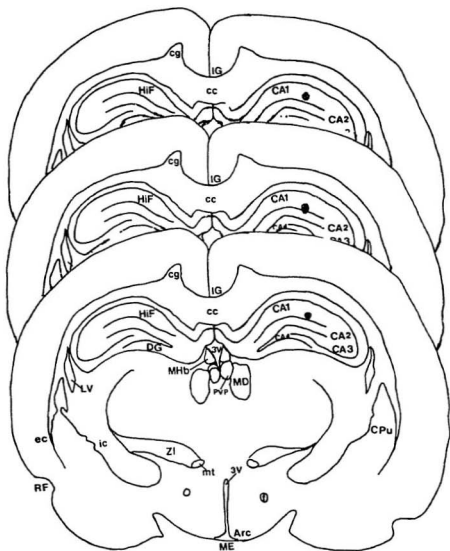


Fig. A7 shows the serial camera lucida drawings of the extent of lesion at the electrode tip recording site. The slices shown here are 80 um apart. The lesion was confined mostly to the CA1 as shown by the dark area in the hippocampus.

Legends: Arc- Arcuate hypoth. nuc., CA1, CA2, CA3, CA4- Fields of Ammon's horns, cc- Corpus callosum, cg- cingulum, CPu- Caudate putamen, DG- Dentate gyrus, ec- Ext. capsule, f- fornix, HiF- Hippocampal fissure, ic- Internal capsule, IG- Induseum griseum, LV- Lateral ventricle, mt- Mammillothalamic tr., MD- Mediodorsal th. nu., ME- Median eminence, MHb- Med. habenular nu., PVP- Paraventricular th. nu, post, RF- Rhinal fissure, ZI- Zona incerta.

1296
376



Bregma -3.3mm



